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Effect of hypoxia on cancer progression

Hypoxia is a condition in which the oxygen level reaching the tissues is lower than normal, as opposed to anoxia which is the complete lack of oxygen.

Normal cells obtain most of their metabolic energy from glucose metabolism through glycolysis. Glycolytic breakdown to pyruvate results in a net production of two ATP and two molecules of NADH. The pyruvate is then converted into acetyl Co-A which enters the TCA (Krebs) cycle and is stepwise degraded in the mitochondria which by the process of oxidative phosphorylation generates a further 36 molecules of ATP (1). In conditions where oxygen is depleted (hypoxia) whether temporary or long term, pyruvate will not be converted to acetyl Co-A and it will not enter the Krebs cycle but instead it will be diverted into lactate (2). The effect of hypoxia on a tissue can be either positive or negative depending on the severity and duration. Although hypoxia is thought to be a pathological phenomenon it is a necessary stimulus that is required for the appropriate function and patterning of most organs of the mammalian embryo (3). Physiologically, hypoxia can arise during short bursts of strenuous physical activity requiring rapid production of ATP during which oxygen supply to muscle tissue may be impaired (4).

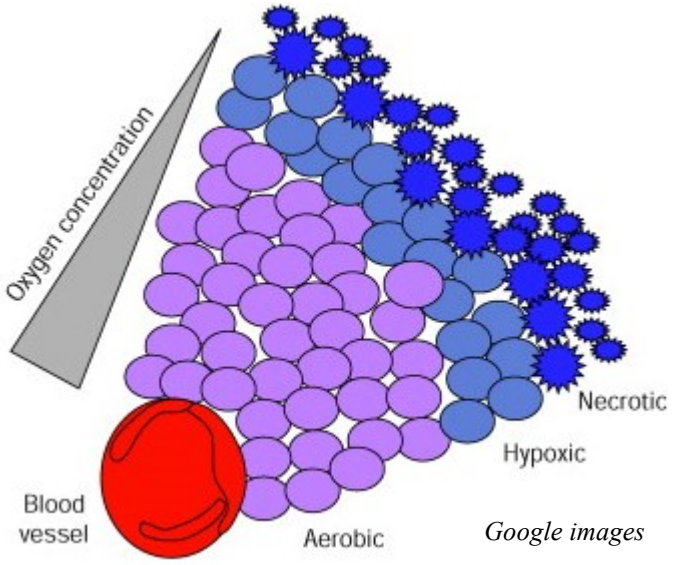
Hypoxic tumor cells acquire their energy (ATP) from glycolysis and even if oxygen is restored they still continue with the same process rather than reverting back to oxidative phosphorylation as do normal cells; this is called the Warburg effect. This phenomenon, along with dysregulated metabolism is a common feature of tumours.

Warburg effect

Longer term hypoxia is usually detrimental to normal cell functioning, since it reduces ATP generation and increases lactate production (5). In 1920, Otto Warburg observed that tumors showed high glucose dependence and lactate accumulation. He suggested that these cells preferred to generate ATP through glycolysis alone (2).

It is a common feature in most malignant tumors though it varies between tumor types (6). Tumor cells adapt to this condition and seem to prefer metabolizing glucose to lactate and derive more of their energy from that; consequently, the high glucose requirement (Figure 1).

To maintain the high rate of glycolytic metabolism in tumors, and their proliferation capacity, the product G-6-P rapidly distributes primarily across two key metabolic routes; (a) entry of G-6-P into the pentose–phosphate shunt for biosynthesis of nucleic-acid precursors, and (b) conversion of the G-6-P via the glycolytic pathway to pyruvic acid. Most of the pyruvic acid is reduced to lactic acid and transported out of the tumor cell via lactate transporters. This promotes an unfavorable environment for the surrounding normal cells with concomitant regeneration of NAD^+ within the cells to maintain glycolysis. Some pyruvate is directed to mitochondria across VDAC (voltage dependent anion channels) and via "as-yet-uncharacterised" pyruvate transporter on the inner mitochondrial membrane. This provides substrates for the TCA cycle for energy generation, as well as lipid and amino acid biosynthesis.



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This feature of cancer cells is utilized for clinical detection of metastatic deposits using radioactive glucose analogues such as fluorodeoxyglucose (FDG) with positron emission tomography (PET)(7). Cancer cells form lactate from pyruvate by increasing the expression of lactate dehydrogenase A and by inactivating pyruvate dehydrogenase through anaerobic

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glycolysis even though oxygen is present. Also, there is an increased production of lactate through enhanced rate of glycolysis. It is an adaptive response which shows that tumor cells prefer to do their metabolic activity with apparently inefficient energy output (8). This occurs despite still having functioning mitochondria though in reduced quantity and capacity (9).

Hexokinase 2 which phosphorylates glucose and tags it for glycolysis is pivotal in initiating the Warburg effect. Hexokinase 2 is bound to the mitochondria and is greatly overexpressed in cancer cells causing the increased rate of glycolysis (10, 11). Additionally, the isoform of pyruvate kinase, PKM2, has been shown to play a role in the metabolism of cancer cells. PKM2 is at the terminus of the glycolytic pathway dephosphorylating (phosphoenolpyruvic acid (PEP) conversion to pyruvate. PKM2 has less enzymatic activity than PKM1, which is more commonly found in normal cells. It constitutes a bottle neck that channels glycolytic intermediates into anabolic pathways and biosynthesis (12).

There is a major metabolic shift in cancer cells that occurs at multiple steps in glycolysis. The primary reason why these metabolic changes may be advantageous to cancer cells is because the switch allows some of the pyruvate generated to go towards biosynthesis, which is accelerated in rapidly multiplying cells (13). Indeed, it has been suggested that cancer may be better thought of as a metabolic disease rather than a genetic one.

This is exemplified by a study in which a tumor cell nucleus was transplanted into the cytoplasm of a normal cell and the cell did not become malignant. On the other hand, a normal cell nucleus transplanted into a tumor cell, resulted in production of tumor cells. The state of the daughter cell was dependent on the contents of the cytoplasm and not the nucleus. This points to metabolic dysfunction, which plays a major role in cancer progression (14).

Glycogen metabolism in tumor hypoxia

In terms of energy balance, cancer cells have increased glycogen storage that is pivotal in glucose metabolism (15,16). Glycogen storage is emerging as a metabolic survival pathway (15). It has been suggested that hypoxia acts as a warning sign for cells, to predict extreme nutritional distress, that aids in their survival (17). Several studies have been conducted to show how hypoxia affects glycogen accumulation. All the cells tested had detectable increased levels of glycogen after

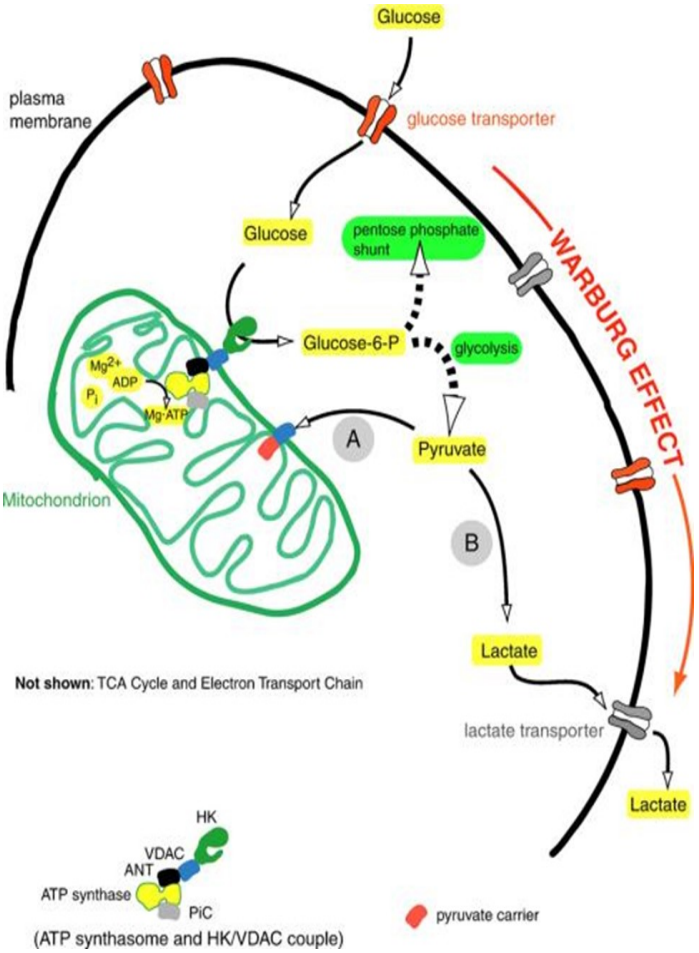

Figure 1. Warburg effect

incubation for 96-h in hypoxia, with the exception of PC3 (Human prostate carcinoma) cells. RCC4 (Human renal clear carcinoma) cells had the largest amount of glycogen (15).

Glycogen storage in cancer and non-cancer cell lines and the role of HIF

One study showed that under normoxic conditions, CCL39 (Chinese hamster lung normal fibroblast) cells and LS174 (Human colon carcinoma) cells did not show any glycogen particles. However, during hypoxia these cells accumulated intensely stained mono-particulate forms of glycogen in their cytoplasm. Pelletier et al. (2012) investigated the role of hypoxia induced factor (HIF) in glycogen accumulation using Human renal clear carcinoma (RCC4) cells that contained stable HIF 1a and HIF 2a, and 786-O cells with stable HIF 2a only due to a defect in pVHL. This is a tumor suppressor that degrades HIF-a proteins. They examined RCC4 cells in which wild type VHL was re-introduced and by microscopy observed that no glycogen was present in RCC4 (+pVHL) cells because HIF-a was degraded. However, large aggregates of glycogen particles were visible in RCC4 (-pVHL) cells under normoxic conditions. To confirm these results, they

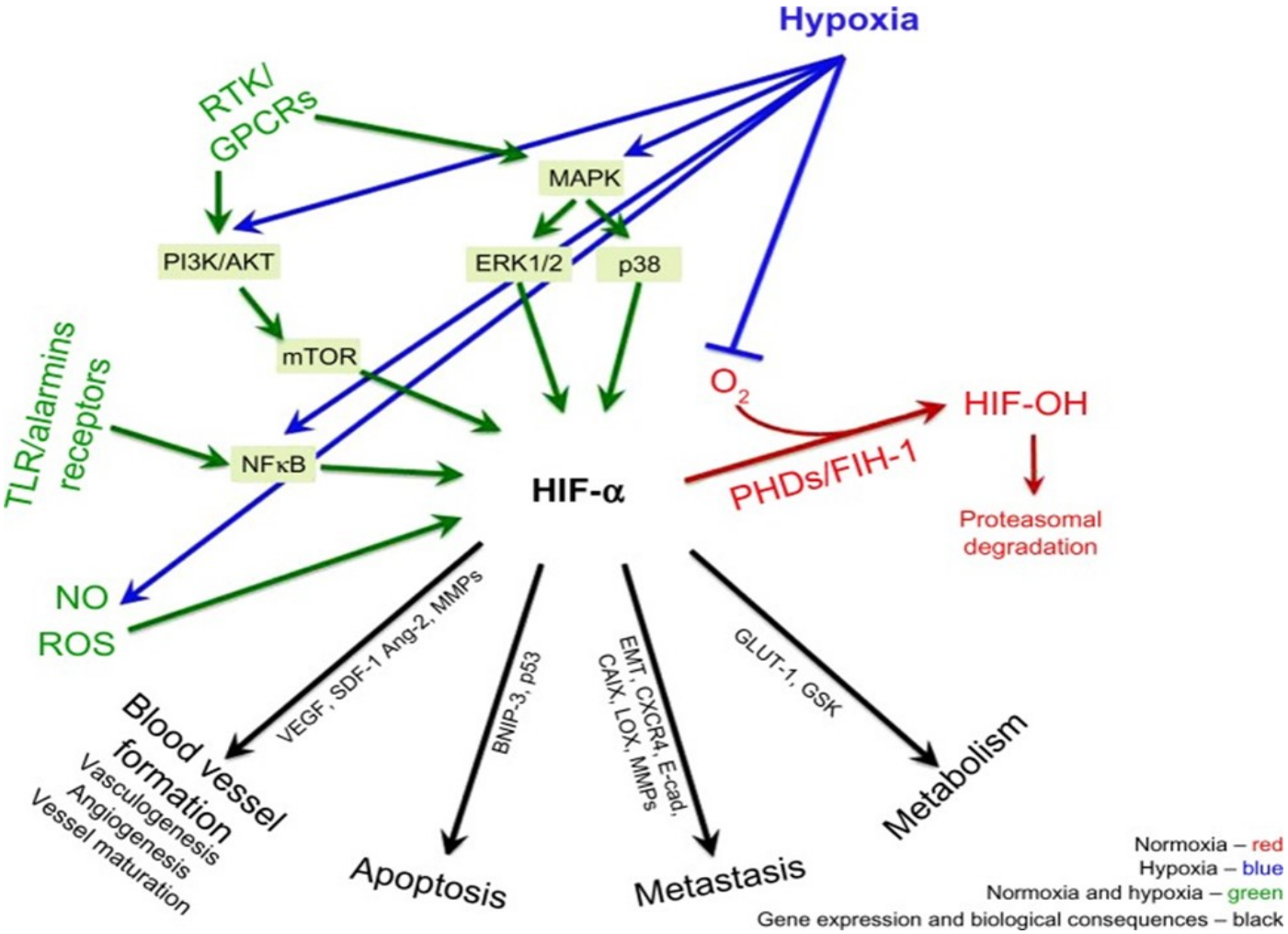

Figure 2. Regulation of HIF in normoxic and hypoxic conditions

measured glycogen accumulation in RCC4/786-O (-pVHL) cells and found a high concentration of glycogen compared to RCC4/786-O (+pVHL) cells. They concluded that hypoxia induced glycogen accumulation is predominantly dependent on HIF-1a and much less on HIF-2a.

Differential time-dependent induction of G6PD gene by hypoxia

During hypoxia, the ribose-5-phosphate was identified as being one of the most strongly up-regulated metabolites and in some cancer cells G6PD was also up regulated (9). A study was conducted on mouse embryonic stem cells with deleted G6PD gene. These cells failed to produce NADPH in response to oxidative stress, which resulted in decreased GSH that led to cell death (20). ROS such as hydrogen peroxide activates G6PD in response to oxidative damage and when this G6PD is over-expressed, it will suppress hydrogen peroxide induced cell death (18,19).

Signaling pathways related to tumor hypoxia HIF pathway

Cellular adaptation to hypoxia is mediated by HIF pathway activation causing uncontrolled tumor metabolism. Likewise, uncontrolled metabolism promotes the activation of HIF (6,9) (Figure 2).

Multiple tumor suppressors and oncogenic pathways activate the HIF system. The most important is the von Hippel-Lindau tumor suppressor (VHL). HIF-α is a transcription factor that can be regulated by both hypoxic and normoxic conditions.

In normoxic conditions, the HIF-a subunits are hydroxylated by oxygen sensors such as PHD and FIH, which are oxygen dependent enzymes causing poly-ubiquitination and proteosomal degradation. In decreased oxygen concentration, these enzymes lose their activity, preventing the hydroxylation of HIF-α, thereby preventing degradation. This non-hydroxylated HIF-a subunit becomes stable, translocates to the nucleus and dimerises with the HIF-β subunit, binds to DNA and initiates transcription of about 100–200 genes associated with erythropoiesis, angiogenesis, autophagy and energy metabolism (20-22).

PI3K/AKT/mTOR (23), MAPK (ERK pathway)

(24), and the NFkB (25) signaling cascade are other hypoxia associated pathways that are activated by mutation and gene amplification (9). These pathways cause unfavorable biological consequences such as cell proliferation, survival, apoptosis, metabolism, migration and inflammation. The dysregulation of the PI3K/AKT/mTOR pathway increases the synthesis of HIF-α subunits which leads to up-regulation of HIF.

The AKT has multiple downstream targets, such as the mTOR and GSK3β. Both the mTOR dependent and independent mechanisms increase HIF-a translation, while the GSK3β is pivotal in regulating HIF-1α protein degradation through a pVHL independent mechanism (26, 27). The MAPK pathway affects the HIF activity by regulating the transactivation process (24).

Another pathway involves the p53 tumor suppressor which once activated, suppresses HIF activity (9). These pathways are also stimulated in hypoxia in an independent manner by a ROS, nitric oxide (NO), chemokines, cytokines, lipopolysaccharides and growth factors that bind to the tyrosine kinase receptor.

Toll-like receptors (TLR), G protein-coupled receptors (GPCR), and alarmins receptors on the cell surface may also lead to HIF-1a activation. Also, acquired mutations in these pathways may lead to over-stimulation of the receptors that leads to uncontrolled cancer cell proliferation (6).

The non-hypoxic HIF regulating pathway that has many signaling cascades including NFkB, PI3K/AKT/mTOR, MAPK, and ROS production, resulting in multiple levels of HIF-α stimulation in both hypoxia and normoxia.

Epigenetic changes and mutations regulate the stability and activity of HIF-α, leading to a gain in oncogenic activity (Ras, Raf, mTOR, Src, Myc) and a loss of the tumor suppressor functions (PTEN, VHL, p53, ING4) (28,29). HIF-α accumulation activates genes such as VEGF and SDF-1 that facilitates angiogenesis, GLUT-1, GLUT-3 and glycolytic enzymes to regulate metabolism, apoptosis and cell survival by means of BNIP-3, p53, and TGF-β and tumor metastasis by altering adhesion and motility by regulating epithelial to mesenchymal transition (EMT) and E-cad expression.

Also, migration and invasion potential is mediated through CXCR4, CAIX, LOX, MMP-2 and MMP-9 (30, 31).

When different signaling pathways are activated at the same time and target the same genes, this is because of the diverse characteristics of tumors, such as hypoxic and inflammatory phenotype.

The malignant and metastatic phenotype of tumour cells is controlled by HIF-1a and NFkB leading to:

- Enhancement of tumor cells survival through many growth factors, and inhibition of pro-apoptotic pathways
- Tumor neovascularization promoted by VEGF
- Downregulation of the adhesion molecules (cadherins) controlling cell detachment
- Induction of cell migration and invasion by matrix degrading enzymes (32).

The endogenous markers for tissue damage and necrosis such as alarmins activate NF-κB in the presence of hypoxia. HIF-1α upregulates the expression of receptors for advanced glycation endproducts (RAGE) and toll-like receptors (TLR) that recognise these markers for activation (6).

PI3K

The PI3K/AKT/mTOR (mammalian target of rapamycin) pathway is a survival pathway that is vital in many cellular functions such as proliferation, migration, invasion, metabolism, angiogenesis. It is activated by loss of PTEN function, amplification, over-expression or mutation of PI3K and AKT and activation of EGFR. This pathway is initiated at the cell membrane in many tumor cells (33). Increased angiogenesis and tumorgenesis are characteristics of endothelial cells that are PTEN deficient leading to poor prognosis and reduced survival in cancer patients (34-36).

Akt (Protein kinase B)

Akt, a serine/threonine specific kinase, also called protein kinase B (PKB), is the central modulator of the PI3K that is found in most tissues in the form of similar structural isoforms, such as Akt-1, -2 and -3 (29, 37). It promotes many downstream substrates, one of which is the direct phosphorylation of the mTOR, a master regulator of protein synthesis and cell proliferation. Therefore, structurally and functionally abnormal blood vessels are formed when Akt is activated in endothelial cells (33).

NFκB

HIF-1α expression is directly mediated by NFκB (nuclear factor κB), a transcription factor that exists as a hetero- or homo-dimer; some are more ubiquitously expressed than others. These dimers alternate between the nucleus and cytoplasm although they are mainly sequestered in an inactive state in the cytoplasm. Endogenous NFκB can be stimulated by compounds such as TNFα (tumor necrosis factor α), oncogenes and UV light that change the levels of HIF-1a in an NFκB dependent manner.

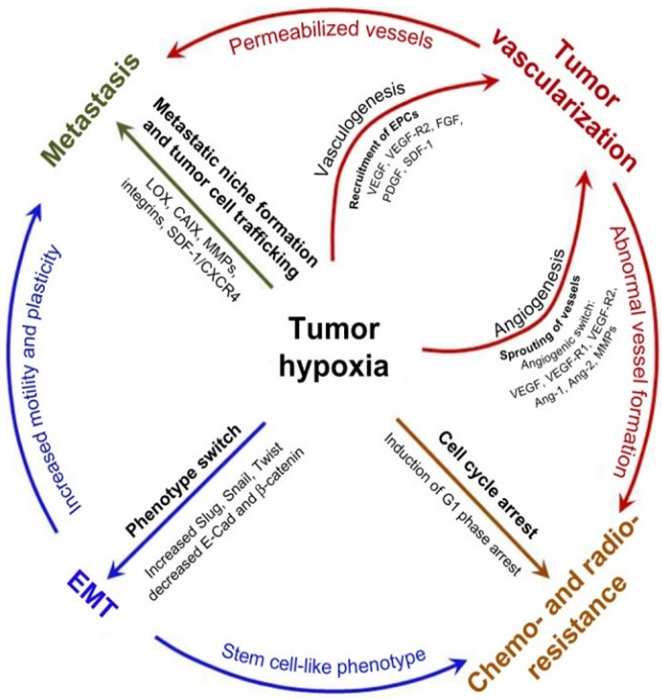

Figure 3. Hypoxia as a driving force of tumor progression and metastasis

Once stimulated a kinase signaling pathway phosphorylates IκB (inhibitor of NFκB) causing ubiquitination that leads to protein degradation and NFκB becomes stable and translocates into the nucleus to bind to target DNA sequences leading to NFκB regulated genes transcription (28). Activation of NFκB is a well-controlled and rapid process that eases the control of many genes and that once depleted leads to reduced levels of HIF-1a (38).

Disorganised blood vessel formation and inadequate tumor cell auto-regulation cause regions of hypoxia due to interruptions in blood flow within solid tumors. This in turn increases the expression of HIF-1α, leading to enhanced aggressiveness and poor survival (39). However, one theory proposes that hypoxic tumors are more aggressive than indolent tumors, which causes them to outgrow their blood supply, indicting HIF-1a as a marker for malignancy rather than a causative factor (22).

Role of hypoxia in cancer progression

The consequences of HIF-1a overexpression leads to increased blood vessel formation, metastasis, aggressiveness and resistance to treatment (Figure 3):

i. Blood vessel formation

This hyperproliferative state is a characteristic of tumor cells in which new blood vessels are formed. This maintains the blood flow to the tumor and provides nutrients and oxygen for the growing cancer cells. Consequently more cells means more demand that then exceed their blood supply eventually leading to a hostile hypoxic environment. To decrease or fix this vicious cycle, tumor cells stimulate more and abnormal angiogenesis (6). HIF-1α is responsible for recruiting endothelial progenitor cells (EPC) from the bone marrow and by regulating the VEGF, a regulator of vasculogenesis, induces their differentiation into endothelial cells (40, 41). It also induces the expression of VEGF-R1, Ang-1 and Ang -2 that regulate endothelial cell proliferation and MMPs that sprout and split the pre-existing vessels. Supporting blood vessel maturation to form stable and mature blood vessels is induced by Ang-1, PDGF, and TGF-β to recruit smooth muscle cells and pericytes (42). However, neo-vessels in tumors can be either insufficient or excessive and are frequently abnormal, immature, and leaky, resulting in a dysfunctional vasculature (43).

ii. Metastasis

Tumor metastasis correlates with poor survival because it is associated with aggressiveness of many cancers. These tumor cells escape the unfavorable hypoxic environment by extravasation, circulation and relocation (metastasis) to unaffected tissues through a heterogeneous dysfunctional vasculature (6, 44). Azab et al. (2012) showed that when multiple myeloma cancer cells were cultured in hypoxic conditions and then injected into mice, they were able to spread to the bone marrow more rapidly than those cultured in normoxic conditions.

iii. EMT

It is characterized by a decrease in epithelial gene expression, such as E-cad and β-catenin and an increase in mesenchymal gene expression, such as N-cad, vimentin, SMA, and CXCR4. EMT is pathologically active during carcinogenesis in many types of solid tumors and hematologic malignancies. Radio- and chemo-resistance have also been observed in the EMT phenotype (6, 30).

iv. Treatment resistance

Slowly proliferating cells, decreased senescence, chaotic and malfunctioning blood vessels and increased metastasis are the consequences of hypoxia which induces further resistance to therapy. Cellular adaptation of tumor cells to hypoxia leads to drug resistance by inducing quiescence, which is a state of reduced cell proliferation protecting the cells from stress and chemotherapy (45, 46, 47). The majority of radio- and chemo-therapies require oxygen for their activity. The normoxic cells are sensitive to ra-

diation in the presence of O_2 leading to irreversible DNA damage. However, in hypoxia, irradiated tumor cells become resistant to death because ROS generation is reduced, sequentially decreasing DNA damage. It was shown that anti-cancer treatments preferentially target rapidly proliferating tumor cells, compared with quiescent and slowly-proliferating ones (47-49).

Mechanism of pH control

The accumulation of lactate on the outer membrane of cancer cells creates an acidic extracellular pH (6-6.9) which is different from the extracellular pH of normal cells (7.3-7.4). This extracellular acidification leads to an aggressive, metastatic and chemo-resistant phenotype. Manipulating pH is being explored by administration of alkalinising buffers as a potential therapy. As already discussed, the intensive utilisation of glucose by cancer cells produces excessive protons and lactate, which lowers the intracellular pH. In order to prevent intracellular acidosis, lactate is extruded resulting in a lower extracellular pH than is usually seen around normal cells. This process involves a number of membrane transporters (50):

i. ***Carbonic anhydrase (CA):*** The most extensively overexpressed CAs are 9 and 12 (51). CA9 can be an independent prognostic indicator for metastasis (52). This enzyme changes the CO_2 to HCO_3 and H^+. HCO_3 then goes back into the cell through Na^+-driven Cl^-/HCO_3^- exchanger (NDCBE) and pushes out the proton through the cell membrane thereby maintaining increased intracellular pH and extracellular acidity (53).

ii. Vacuolar-type H^+ ATPases (V-ATPases): this is one of three types of ATP H^+ driven pumps that transports protons into the extracellular space across the plasma membrane to maintain an alkaline pHi and acidic pHe (54). In tumor cells, this transporter contributes to invasion and metastasis leading to drug resistance by neutralizing drugs extra or intracellularly, decreasing drug internalization and inhibition of apoptosis (54).

iii.Monocarboxylate transporter (MCT): MCT4 transports excess lactate out of hypoxic cells into the extracellular space where it may be subsequently taken up by nearby cells in normoxic conditions via MCT1 found on these cells. This lactate is then utilized as a substrate for conversion to pyruvate and entry into the TCA cycle to generate ATP (55). Tumor cells often express both MCT1 and MCT4 that are associated with CD147, an extracellular matrix metalloproteinase inducer that facilitates tumor cell invasion (56).

iv. Sodium/hydrogen exchanger (NHE): this is an antiporter in the plasma membrane that balances the pH by the exchange of H^+ for Na^+ (Na^+ influx and H^+ efflux). When pHi reaches a certain point, NHE becomes inactivated even in the presence of a large amount of sodium (57). NHE-1 is ubiquitously expressed and plays an important role in regulating pHi (58).

v. Bicarbonate transporter family (BCT): These regulate pHi through three transporters, Na^+ dependent and independent Cl^-/HCO_3^- exchangers and $Na^{+/}HCO_3^-$ co-transporters. NDCBE prevents intracellular acidification by extruding Cl^- in exchange for $Na^{+/}HCO_3^-$ complex. However, the Na^+-independent transporter prevents intracellular alkalization by HCO_3^- efflux and Cl^- influx (55).

Manipulation of tumor pH as a potential therapeutic strategy

Arterial vasodilators such as hydralazine may selectively increase the perfusion to normal tissues leading to cardiac output redistribution away from the tumor, thus selectively decreasing tumor blood flow, which consequently reduces tumor pH. Some may argue to not use this vasodilator in combination with chemotherapy as it may lead to less drug concentration at the tumor site because of decreased blood flow (59).

Manipulating pH will alter chemotherapy effectiveness depending on the type of drug and pH. If the chemotherapy agent used is a weak base (e.g. doxorubicin, daunorubicin, gemcitabine, mitoxantrone, epirubicin, idarubicin, valrubicin, bleomycin, vinblastine), the drug will be deactivated by the low pH before entering the tumor cell. So using drugs to increase pH (less acidity) can help increase their effectiveness. While using weakly acidic drugs (cyclophosphamide, carboplatin, mitomycin C, melphalan) at acidic pH will enhance their activity and lead to cytotoxicity (59,60). Altering pHe could kill tumor cells, reverse drug resistance and reduce cancer metastasis.

Chemotherapeutic agents are occasionally non-selective against cancer cells resulting in many side effects. We can use strategies to reduce or inhibit major acidity exporters and eventually alter

the acidity of the tumor microenvironment:

i. V-ATPase inhibitors

High doses of proton pump inhibitors (PPI) can increase the sensitivity of tumor cells to cytotoxic molecules. Wang et al. (2015) showed that the antitumor effect of chemotherapy was increased using intermittent high doses of PPI and there was no evidence of additional toxicity. PPIs can enhance the effectiveness of immunotherapy and spontaneous antitumor immune response (61). The activity of weakly basic drugs (doxorubicin or metoxantrone) was increased in a mouse breast cancer model when pre-treated with HCO_3^- (62). A class of PPI (esomeprazole, omeprazole, lansoprazole) pro-drugs are administered orally or systemically and they need an acidic pH to be transformed into the active compound. For example, omeprazole at low pH is converted to its active form sulfenic acid (63) and has shown promising potential as a future anti-cancer drug (64). These weakly basic pro-drugs are selective inhibitors of gastric H^+K^+ ATPase and V-ATPase that can penetrate the cell membrane easily due to their lipophilicity. Absence of general toxicity in this class of pro-drugs is due to their dependence on the acidic pH therefore activating cytotoxic metabolites (65). PPI's can cause direct toxicity by potentially reducing tumor acidity, depriving them from the key condition for their survival. They are recommended for phase III clinical testing (66).

ii. CA9 inhibitors

The position of CA9 on the extracellular surface of the cell makes it an effective target for CA9-specific cancer therapies, including small molecule inhibitors and monoclonal antibodies (mAbs). Immunotherapy using CA9 specific mAbs exerts its anti-tumor effect by binding directly to CA9, leading to antibody mediated cell cytotoxicity. Also, receptor mediated internalisation allows the targeted delivery of cytotoxins and radionucleotides (67). M75 and G250 are CA9 specific immunological tools that can be used for both clinical detection and treatment. Immunohistochemical staining of CA9 in tumor cells can be detected by M75, a highly specific antibody. A radiolabeled form has been developed to be used for imaging CA9 but not as an anticancer immunotherapy (68,69). However, a chimeric version of the G250 (cG250) has been developed and characterized as an immunotherapeutic anti-tumor agent that induces antibody dependent cellular cytotoxicity alone or in combination with interferons (IFN-α) (70,71). Small molecule compounds such as sulfonamides and coumarins that are known to effectively inhibit CAs have shown promising potential as anti-cancer agents (72). Sulfonamides coordinate with the zinc ion within the active site to inhibit CA9, while the coumarins, also known as suicide inhibitors, undergo hydrolysis to 2-hydroxycinnamic acids that bind irreversibly to the active site of CA (67,73).

iii. Na^+/H^- exchange (NHE) inhibitors

Amiloride and its derivatives and guanidine and its derivatives (benzoylguanidine, carbonylguanidine) are two classes of NHE inhibitors. Amiloride non-selectively inhibits all NHE isoforms. Moreover, apoptosis of leukemic cells can be induced by amiloride derivatives, although there is a difference in the sensitivity of normal and leukemic cells to these agents, suggesting that NHE1 inhibitors may be used as anti-leukemic agents. Zoniporide (carbonylguanidine) may be used therapeutically as a cardioprotective agent (58).

iv. MCT inhibitors

Aromatic monocarboxylates, inhibitors of anion transport (α-cyano-4-hydroxycinnamate), and bioflavonoids (quercetin) are three classes of MCT inhibitors (58). Normoxic tumor cells become less dependent on anaerobic glycolysis when MCT1 and MCT4 are inhibited, thus relying on aerobic respiration to generate ATP, which causes glucose starvation resulting in the death of hypoxic tumor cells (74).

v. BCT inhibitors

DIDS and SITS are non-selective inhibitors of the BCT transporter. Triflocin and S3705 are selective inhibitors that inhibit the Na-HCO_3 symport in the proximal tubule and inhibit the NDCBE activity and tumor growth, respectively (58).

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Ann Hashem
Final Year student
Faculty of Pharmacy,
Kuwait University

TEST YOUR KNOWLEDGE

Answers on back page

1) Which of the following is a transcription factor that directly mediates HIF-1α expression?

A. NFκB
B. BhLH-PAS
C. EPAS
D. NTAD

2) The drug which is a weak base and is deactivated by low pH before entering the tumor cell is:

A. Cyclophosphamide
B. Mitomycin
C. Doxorubicin
D. Carboplatin

3) Which of the following is a NHE inhibitor that can induce apoptosis of leukemic cells?

A. Amiloride
B. Omeprazole
C. Metoxantrone
D. Oximidine

Is there a problem?
A 45 year old male patient was given the following prescription for his newly diagnosed hypercholesterolemia. Is there any major error with the prescription?

BDX HOSPITAL

Patient Name: Mr. Ahmad Age: 45 years
Address: Street No.33

Rx

Rosuvastatin 20 mg tablet
Once daily
Send one pack

Dr. Michael
Signature Date: 28/09/17

Answer (Prescription Exercise)

The initial dose is wrong. It should be 5-10 mg once daily, increased if necessary at intervals of 4 weeks, to 20 mg once daily.

Source: British National Formulary

TOPICAL ISSUES AND CONTROVERSIES

Sugar: Leaving a bad taste in the mouth

The debate over sugar is heating up. Initially perceived as innocuous, sugar is increasingly coming under the scrutiny of health officials globally, as new studies point to a strong link between excess consumption and the twin epidemics of obesity and diabetes type II.

A report shows that over the past 30 years, the global average daily sugar consumption per person has risen 46 percent to the equivalent of 17 teaspoons, which is nearly double the maximum level recommended by the American Heart Association. The worst offenders are the U.S., Brazil, Argentina, Australia and Mexico, which consume more than double the world average.

The surge in intake of added sugar - sugar not found in natural products, like fruit or milk- has coincided with a significant increase in the rates of obesity and diabetes, which currently affect nearly 900 million people around the world and cost the global healthcare system more than $1 trillion a year.

The statistics have certainly caught the eye of the $24 billion global sugar industry, as growing public concern about the health effects of sugary drinks and the ballooning cost of treating the associated diseases ramp up pressure on authorities and companies to take action.

Sugar-sweetened drinks, rather than processed

foods, are in the sights of anti-sugar campaigners because of the way the body processes liquid calories. Sugar in soft drinks is completely digested by the body without the consequential satiation of appetite that happens with solid food. In addition, sugary beverages account for over 40 percent of the added sugars we ingest.

But despite the huge social and economic impact of obesity and diabetes, governments and health officials only recently started to introduce measures aimed at curbing sugar consumption, such as anti-soda advertising campaigns and bans on vending machines in schools.

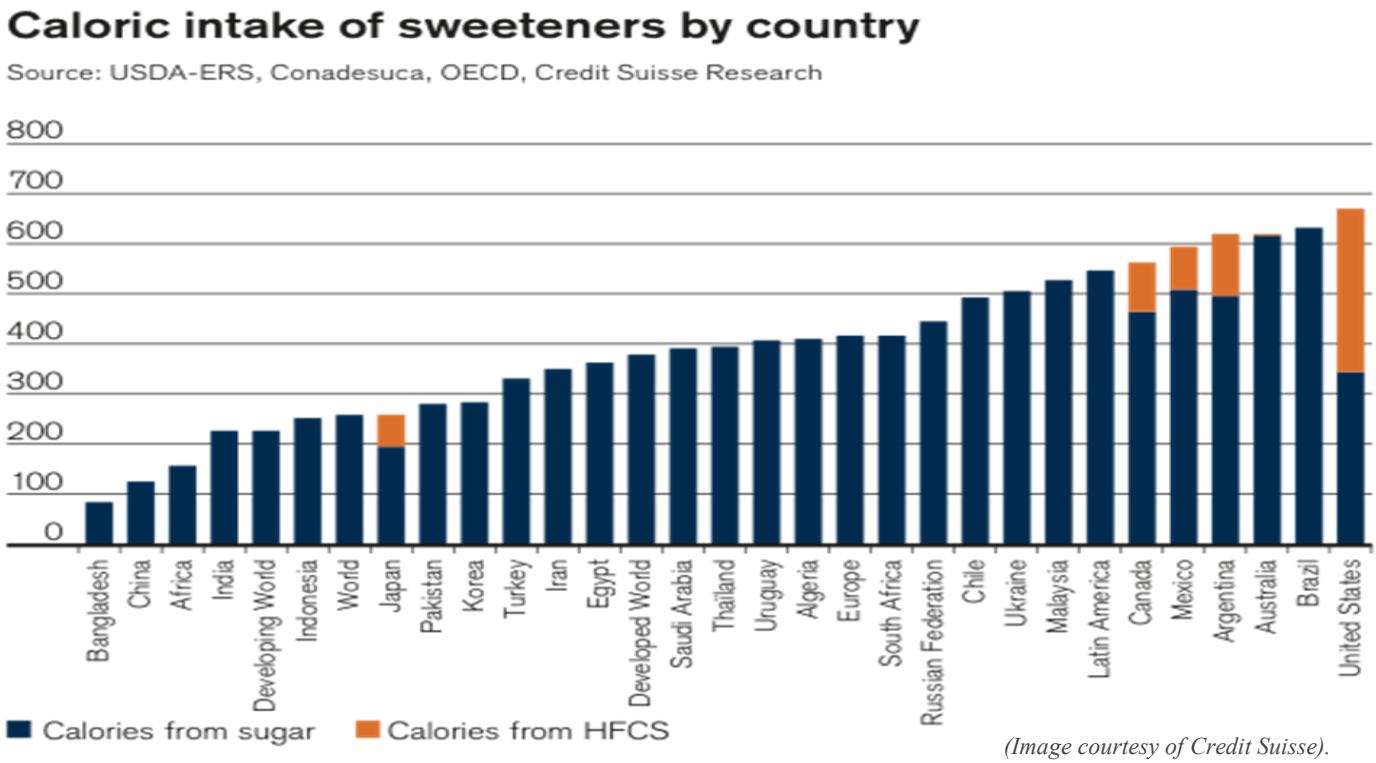
effective. Pointing to the impact of taxation on consumption of tobacco and alcohol, they predict that even the mere threat of taxes would drive companies to self-regulate and take concrete actions to reduce the amount of added sugar in their products.

But the debate about taxing sugar-sweetened soft drinks is divided along health and socio-economic lines. Support is highest among underweight and normal weight people, as well as those with the highest income and education level.

Taxation has proven effective in France, where a 2 euro cent tax on sugary and artificially flavored drinks -equivalent to nearly 5% of the overall value- reduced the carbonated soft drink market by 5% in terms of volume. In Mexico, one of the world's biggest consumers of soda, legislators are considering a 1 peso per-liter levy on sweetened drinks as part of a financial reform package sent to Congress.

To date, there is no conclusive scientific research proving a causal relationship between sugar consumption and obesity and diabetes type II, but most doctors believe the data supporting the connection is convincing.

A Credit Suisse survey of 152 doctors in the U.S., Europe and Asia found that nearly 90% believed there was a strong link between excess sugar consumption and obesity, based on the patients they see in their day-to-day work. An even greater number, 91%, saw a linkage to diabetes type II.



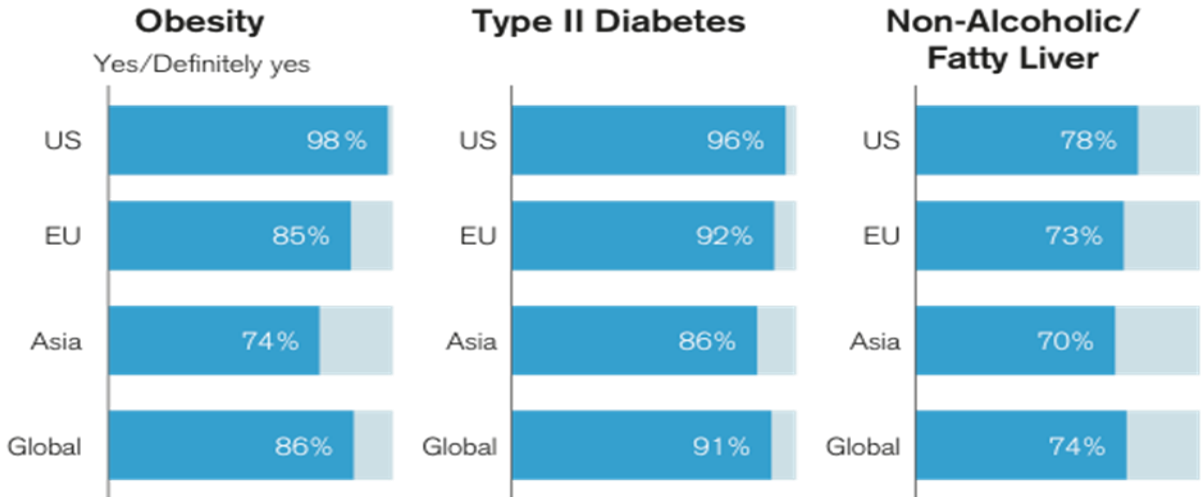
(Image courtesy of Credit Suisse).

Taxing the Sweet Stuff

While there is no silver bullet to reducing sugar demand and reversing the obesity and diabetes type II epidemics, the report said there is plenty of evidence to show taxing sugar-sweetened drinks is

Credit Suisse Equity Research Nutrition survey 2013

Would you say sugar consumption is linked to development of ?



The zero cal response

Large beverage companies such as Coca-Cola and Pepsi are already responding to growing public anxiety about the health effects of sugar, experimenting with natural sweeteners with zero or minimal caloric content to replace sugar and controversial artificial sweeteners in their drinks.

Data shows diet drinks make up less than 10% of the carbonated-drink market in Asia, Eastern Europe, Southern Africa, the Middle East and Latin America. In Western Europe, North America and Australasia- where the average consumer enjoys a higher income and education level- diet drinks account for between 24 and 36% of consumption.

Global sugar demand has been growing at 2% a year for the past decade, but it is likely to slow significantly in the coming 5-10 years as public concerns about the health effects of sugar gain momentum.

Makers of artificial sweeteners such as aspartame, which has suffered from debates over its safety, are also likely to struggle as better-tasting natural alternatives become available. Soft drink companies, on the other hand, have the chance to "come out on the winning side" if they make the right changes to their product offerings. Unlike tobacco and alcohol companies, beverage makers have the advantage of being able to offer a healthier version of their products, i.e. natural non calorie sweeteners.

Full-calorie versus diet carbonated-drink consumption – by region

Source: Beverage-Digest, Canadean

2012	Full calorie share of consumption	Diet share of consumption
Asia	96.9 %	3.1 %
Eastern Europe	96.7 %	3.3 %
Southern Africa	95.8 %	4.2 %
MENA	95.2 %	4.8 %
Latin America	93.0 %	7.0 %
Western Europe	75.5 %	24.5 %
North America	69.0 %	31.0 %
Australasia	64.7 %	35.3 %
Worldwide	85.9 %	14.1 %

Source:
http://www.thefinancialist.com/sugar-leaving-a-bad-taste-in-the-mouth-stefano-natella-credit-suisse/

Why artificial sweeteners make you hungrier

In the past decade, a number of alarming studies in humans and rodents have linked NNS (non nutritive sweetener) consumption with increased appetite and weight gain, as well as an increased risk for developing metabolic disorders. A study in the journal *Cell Metabolism* shows a new pathway in the brain that responds to the sweetness and energy balance in food. Researchers gave fruit flies caloric sufficient diets, so they shouldn't be hungry, and then tweaked the sweetness with artificial sweeteners. Six days later, they gave the fruit flies naturally sweetened diets. Flies that had been exposed to sucralose ate more food and consumed more calories even when they returned to a normal diet compared to flies fed a normal diet all along. If both diets were calorically sufficient, why did flies given sucralose consume more calories?

To find out, the team first looked at RNA sequences in fly heads after 6 days of sucralose exposure and found that 30 transcripts were upregulated, including the fly insulin receptor (InR). Blocking insulin production abolished the appetite-inducing effects of sucralose, suggesting that NNSs such as sucralose promote food intake through the insulin system.

The researchers next examined Neuropeptide F (NPF)–producing neurons that house insulin receptors. (NPF is an ortholog of the human appetite-stimulating neurotransmitter Neuropeptide Y). When the scientists selectively suppressed the InR on NPF-expressing neurons by RNA interference, they blocked the appetite-stimulating effects of sucralose.

When the team knocked down NPF receptors (NPFRs) on sweet-sensing taste neurons known to play a role in the sucralose response, they did not see increased feeding after sucralose exposure. Essentially, sucralose causes the flies to perceive natural sugar as sweeter than it is, and this response is mediated through NPF neurons. Thus, the increased sensitivity to sweetness may be driving the increased feeding behaviors. Other research has shown that fasting animals respond to sugars more strongly than their well-fed counterparts and that NPF is involved in that response.

More research is needed to fully understand the overall impact of artificial sweeteners on health to ultimately shape public health policies.

Reference

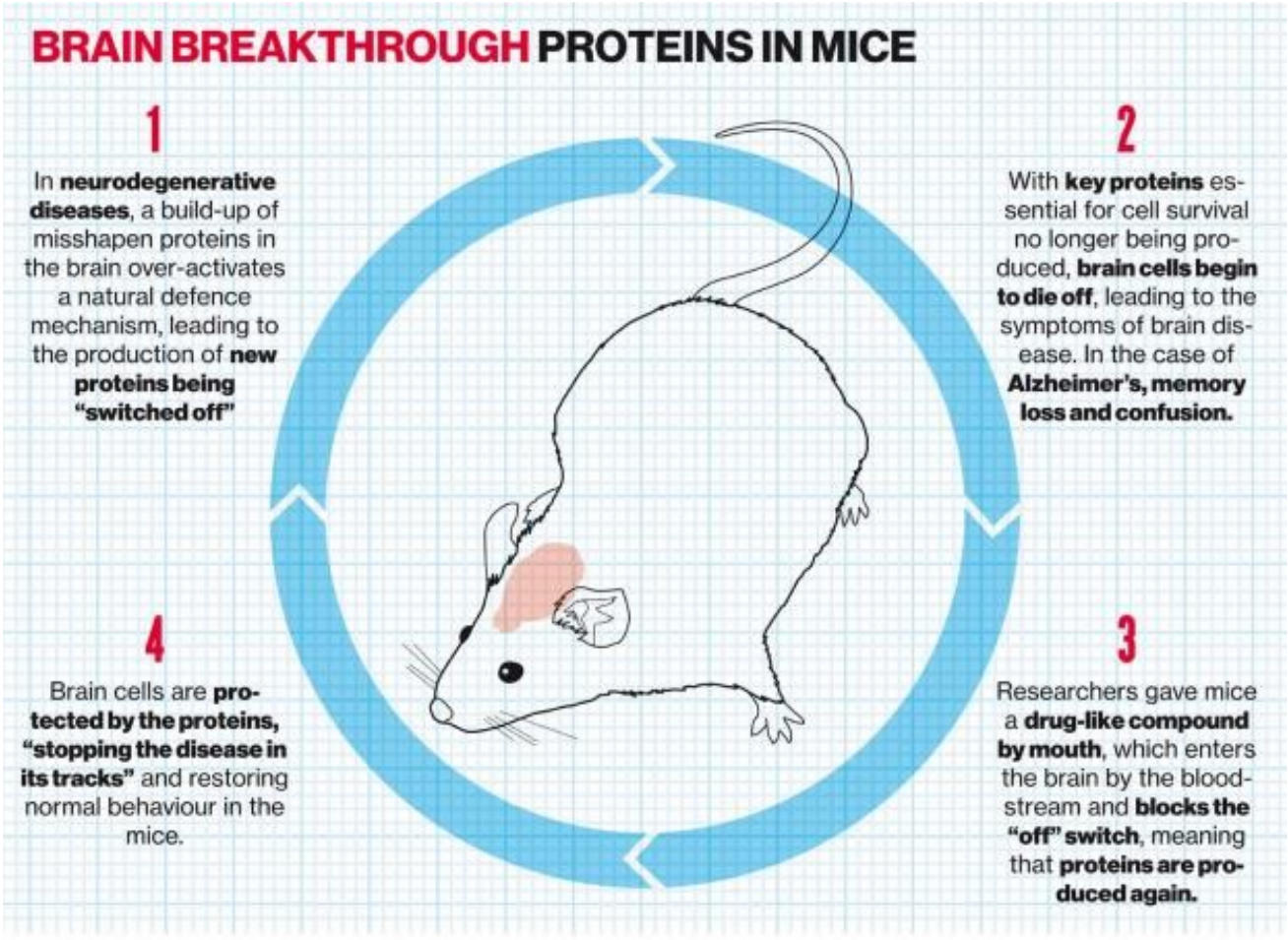
Wang Q et al. Sucralose promotes food intake through NPY and a neuronal fasting response. Cell Metabolism. 2016 July 12; 24, 75-90.

Alzheimer's breakthrough: simple pill to cure disease

There are currently 800,000 people in the UK with dementia and Alzheimer's disease is the most common cause. The number of people living with the condition is set to break one million by 2021, and represents an enormous health burden for the NHS and the social care system. Parkinson's disease affects 1 in 500 people and around 127,000 people suffer from the condition.

Scientists have hailed an historic "turning point" in the search for a medicine that could beat Alzheimer's disease, after a drug-like compound was used to halt brain cell death in mice for the first time. Although the prospect of a pill for Alzheimer's remains a long way off, the landmark British study provides a major new pathway for future drug treatments.

The compound works by blocking a faulty signal in brains affected by neurodegenerative diseases, which shuts down the production of essential proteins, leading to brain cells being unprotected and dying off. It was tested in mice with prion disease - the best animal model of human neurodegenerative disorders, where the same principles would apply in a human brain with debilitating brain diseases such



as Alzheimer's or Parkinson's.

The study, published in the journal, *Science Translational Medicine*, was carried out at the Medical Research Council's (MRC) Toxicology Unit at the University of Leicester.

It is claimed that it is the first time that a compound can be given orally which gets into the brain, and prevents brain disease. It is now possible to go forward and develop better molecules which will translate into brains of mammals. In debilitating brain diseases like Alzheimer's, the production of new proteins in the brain is shut down by a build -up of amyloids. This build-up leads to an "over-activation" of a natural defense mechanism that stops essential proteins being produced. Without these proteins to protect them, brain cells die off - leading to the symptoms of diseases like Alzheimer's.

The compound used in the study works by inhibiting an enzyme, known as PERK, which plays a key role in activating this defense mechanism. In mice with prion's disease, it restored proteins to protect brain cells, restoring some normal behaviors and preventing memory loss.

Although the compound produced significant side effects in mice, including weight loss and mild diabetes, which was caused by damage to the pancreas, it would be possible to develop a drug that protected the brain without these side effects, and that work towards doing so had been "very promising", according to scientists.

The breakthrough was greeted with excitement by scientists, who nonetheless cautioned that it remained a significant proof of principle, and a possible basis for new treatments, rather than a guarantee of an Alzheimer's cure in the near future.

According to experts, targeting a mechanism important to neurodegenerative diseases could give a single drug with considerable benefits, but this compound is still at an early stage. It will be important for these findings to be repeated and tested in models of other neurodegenerative diseases, including Alzheimer's disease. What works in animals does not always hold true in humans, and therefore, the ultimate test will be to see whether this compound is safe and effective in people with these diseases.

Reference:
www.independent.co.uk/news/science/alzheimers-breakthrough-british-scientists-pave-way-for-simple-pill-to-cure-disease-8869716.html

IN THE NEWS

New ASCO guidelines for HER2-negative breast cancer

The American Society of Clinical Oncology (ASCO) has updated its clinical practice guideline on both targeted therapies and chemotherapy treatment for women with HER2-negative breast cancer, which makes up approximately 80% of all breast cancers diagnosed in the United States.


Google images

1. According to the guideline, hormonal therapy, rather than chemotherapy, is the preferred first-line therapy for patients with estrogen receptor–positive metastatic breast cancer, except in cases of immediate life-threatening disease or when a patient is suspected to be resistant to hormonal treatment.
2. Subsequent therapy should consist of sequential chemotherapy not in combination, to reduce adverse events and so as not to diminish quality of life. There is no single agent that is preferred as a first-line or later-line therapy.
3. Clinicians and patients should make treatment decisions together. The decision should be based on patient factors, including prior therapies, toxicity, performance status, comorbidities, and the patient's preference.
4. It was also stressed that the role of bevacizumab for breast cancer is still controversial. Bevacizumab should only be considered with single-agent chemotherapy in the presence of immediate life-threatening disease or severe symptoms. While bevacizumab can shrink tumors and delay disease progression in some patients, the antibody has not been shown to extend overall survival and is not currently approved by the US FDA for the treatment of breast cancer.
5. No other targeted agents should be used in addition to, or as a replacement for, chemotherapy. Only everolimus, a targeted agent against the mTOR pathway, is approved in conjunction with exemestane, a hormonal therapy, for women with early-stage hormone receptor–positive breast cancer, when the disease is still responsive to hormonal therapy.
6. Palliative care should be initiated early and offered throughout the care of breast cancer patients.
7. Since there is no cure for advanced breast cancer, clinicians should encourage all eligible HER2-negative breast cancer patients to participate in clinical trials to potentially benefit from promising experimental treatments.

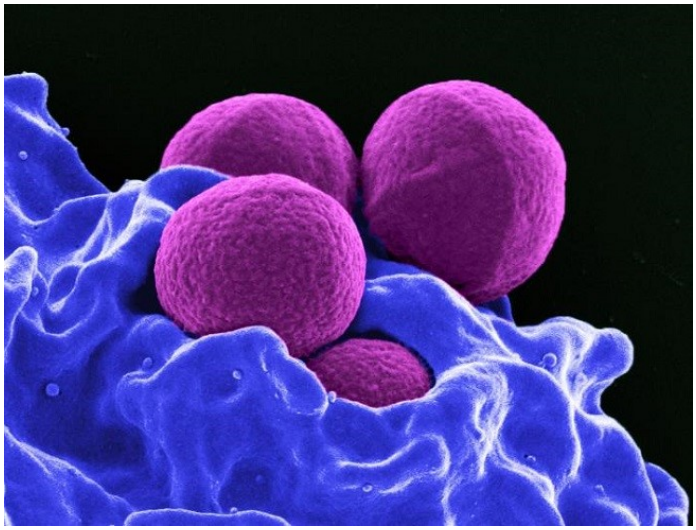
Source: ***http://www.cancernetwork.com/news/asco-updates-guidelines-her2-negative-breast-cancer?GUID=409EAAC3-63BF-4023-8594-***

Antibiotic Assistants: Restoring efficacy against drug-resistant superbugs

Bacteria that have evolved resistance to ß-lactam antibiotics can be rendered susceptible once again if the drugs are combined with newly identified small molecule adjuvants called tarocins.

Researchers at the pharmaceutical company Merck announced their discovery in the *Science* paper showing that a cocktail of ß-lactam antibiotic and tarocin could successfully treat mice infected with a normally resistant strain of *Staphylococcus aureus*.

The authors found a way to re-sensitise resistant bacteria to agents that are effective. Beta-lactams are important antibiotics and being able to get that sensitivity back is very critical. Beta-lactam antibiotics, which include penicillins such as methicillin, kill bacteria by targeting enzymes that are necessary for producing peptidoglycan, the major component of the bugs' cell walls. But some species of bacteria, for example, methicillin-resistant *S. aureus* and *S. epidermidis* (MRSA and MRSE)- have



Resisting the resistance

acquired resistant forms of these enzymes allowing the bugs to proliferate unhindered by the drugs.

MRSA and MRSE are both major causes of difficult-to-treat infections, particularly in hospitals, with MRSA being the second most common cause of death from drug-resistant bacteria. Indeed, the US Centers for Disease Control and Prevention has categorised MRSA as a serious threat.

Recent research has shown that inhibiting the synthesis of teichoic acid- another cell wall component can restore ß-lactam sensitivity to drug-resistant *S. aureus*. However, inhibitors of the teichoic acid pathway identified to date either have eukaryotic toxicity or lack potency.

Merck's researchers therefore carried out a screen of 2.8 million small molecules to try to find safe and effective techoic acid inhibitors. They found two structurally unrelated molecules, which inhibit an enzyme responsible for the first step in teichoic acid synthesis, TarO, naming the compounds tarocin A and B.

The team showed that combining either of the molecules with a ß-lactam antibiotic could suppress growth of both MRSA and MRSE in vitro, and that neither of the compounds were toxic to human cells. The team then created an improved derivative of the more potent of the two molecules, tarocin A, and showed that it could restore ß -lactam susceptibility to 82% of MRSA samples and 77% of MRSE samples isolated from patients. They also showed that mice infected with MRSA could be effectively treated with a combination of ß-lactam and tarocin A with no apparent adverse effects. Treatment with either the antibiotic or tarocin A alone, on the other hand, failed to clear the infections.

That the tarocins lack activity when used alone was not by chance, but design. The team chose to focus their efforts targeting a non-critical part of the teichoic acid pathway. Such an approach is unusual but, it has its advantages. For one, it seems that clinical trials are much more straightforward if the adjuvant is non-bioactive. Compared with an entirely new antibiotic that itself kills bacteria, the regulatory path to clinical development of an inactive adjuvant is faster, easier and less costly.

Another benefit of targeting non-essential pathways, is that it provides scientists with options. This opens up more targets and new chemical space to consider, and one just has to pair it with an existing antibiotic.

Reference:
http://www.the-scientist.com/?articles.list/categoryNo/2901/category/News---Opinion/

Answers to: Test your knowledge

Correct answers:
1-A; 2-C; 3-A

The *Kuwait Pharmacy Bulletin* (ISSN 1028-0480) is published quarterly by the Faculty of Pharmacy, Kuwait University, and includes a list of recently approved drugs from the MOH. It aims to provide instructive reviews and topical news items on a range of drug related issues. It is widely distributed free within the university, to hospitals, polyclinics & private pharmacies as well as to other universities within the Gulf & Middle East region.

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Cover design by Ajwan Behbehani

Editorial Office: Faculty of Pharmacy, Health Sciences Centre, Kuwait University, PO Box 24923 Safat, 13110 Kuwait, Fax:25342087; email: yunus@hsc.edu.kw
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